

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In re Application of

KEITH CHADWICK MURDOCK and
FREDERICK EMIL DURR

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Art Unit 125
Examiner J. D. Goldberg

Serial No. 63,285
Filed August 2, 1979
1,4-BIS(SUBSTITUTED-AMINO)-5,8-
-DIHYDROXY-ANTHRAQUINONES AND
LEUCO BASES THEREOF

GROUP 120

Hon. Commissioner of Patents
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GROUP 110

Sir:

AFFIDAVIT UNDER RULE 132

STATE OF NEW YORK)
) ss.
COUNTY OF ROCKLAND)

ROSLYN E. WALLACE, residing at 27 North Pearl Street, Pearl River, New York 10965, being duly sworn, deposes and says;

THAT she is a trained Biologist, having received the Bachelor of Arts degree in Biology-Chemistry from Brenau College, Gainesville, Georgia in 1944;

THAT she has been employed since 1950 by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York as a Biologist;

THAT she has read and is familiar with the above-identified application for United States Letters Patent and the Office Action thereto, mailed September 9, 1980;

THAT she tested samples of the compounds listed in Tables I-IV according to the following procedures, in her laboratory at the aforesaid Lederle Laboratories Division, Pearl River, New York;

Lymphocytic leukemia P388 test

The animals used are CD₂F₁ mice all of one sex, weighing a minimum of 18 g. and all within a 3 gram weight range. There are 5 or 6 animals per test group. The tumor transplant is by intraperitoneal injection of 0.1 ml. of dilute ascitic fluid containing 10⁶ cells of lymphocytic leukemia P388. The test compounds are administered intra-

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peritoneally on days one, 5 and 9 (relative to tumor inoculation) at various doses. The animals are weighed and survivors are recorded on a regular basis for 30 days. The median survival time and the ratio of survival time for treated (T)/control (C) animals are calculated. The positive control compound is 5-fluorouracil given as an injection at the indicated dose. The results of this test appear in Table I. The criterion for efficacy is $T/C \times 100 \geq 125\%$.

TABLE I
Lymphotoxic Leukemia P388 Test

Compound	Dose (mg./kg.)	Median Survival Time (days)	T/C x 100 (Percent)
Leuco-1,4-bis[(2-dimethylaminoethyl)-amino]-5,8-dihydroxy-antraquinone	200	7	74
	100	25 (2)*	243 (2)*
	50	24 (2)	243 (2)
	25	23 (2)	232 (2)
	12.5	18 (2)	182 (2)
	6	16	160
	3	14.5	145
	1.5	13	130
	0	10 (2)	-
	60	19 (2)	198 (2)
Control			
γ -Fluorouracil			

*In all instances, in this and the following tables, the figures in parentheses gives the number of tests run at that dose level and the figures for Median Survival Time and T/C x 100 are the average of those tests. Where no parenthesis is given, the test was run once at that dose level.

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
1,4-Bis[(2-dimethylaminooethyl)amino]-5,8-dihydroxy-anthraquinone	200 100 50 25 12 6 3 1.5 0.75 0.37 0.18	11 (2) 15 (2) 23 (5) 21 (5) 23 (5) 22 (3) 19 (3) 20 (2)	102 (2) 142 (2) 216 (5) 191 (5) 202 (5) 196 (3) 173 (3) 156 (2) 145 136 109
Control 5-Fluorouracil	0 60 20	12 (4) 29	177 (4) 187
Leuco-1,4-bis(2-morpholinooethyl-amino)-5,8-dihydroxy-anthraquinone	400 200 100 50 25	14 (2) 13 (2) 12 (2) 12 (2)	133 130 (2) 120 (2) 115 (2) 115 (2)
Control 5-Fluorouracil	0 60	10 (2) 17	- 189

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C × 100 (Percent)
1,4-Bis(2-morpholinooethylamino)-5,8-dihydroxy-anthraquinone	400 200 100	11 (2) 12 (2)	105 110 (2) 115 (2)
Control	25	11	105
5-Fluorouracil	0 60	10 (2) 17 (2)	- 174
Leuco-1,4-bis[(2-diethylaminoethyl)-amino]-5,8-dihydroxy-anthraquinone	300 200 100 50 25 12	20 (2) 17 (2) 16 (2) 13 (2) 13 (2)	200 177 (2) 169 (2) 149 (2) 131 (2) 131 (2)
Control	0	10 (2)	-
5-Fluorouracil	60	21 (2)	217 (2)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C × 100 (Percent)
1,4-Bis[(2-diethylaminoethyl)amino]-5,8-dihydroxy-anthraquinone	300	17.5	167
	200	19 (2)	188 (2)
	100	18 (2)	178 (2)
	50	16 (2)	155 (2)
	25	16 (2)	155 (2)
Control 5-Fluorouracil	12	14 (2)	134 (2)
	0	10 (2)	-
Leuco-1,4-bis[[2-(1-pyrrolidinyl)-ethyl]amino]-5,8-dihydroxy-2H-tri-quinone	300	11	100
	200	20 (2)	185 (2)
	100	18 (2)	159 (2)
	50	15 (2)	136 (2)
	25	14 (2)	127 (2)
Control 5-Fluorouracil	12	14	-
	0	11 (2)	-
	0	19 (2)	173 (2)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (days)	T/C x 100 (Percent)
1,4-Bis[(2-(1-pyrrolidinyl)ethyl]-amino]-5,8-dihydroxy-anthraquinone	100	17 (2)	150 (2)
	50	21 (2)	191 (2)
	25	19 (2)	175 (2)
	12	18 (2)	157 (2)
	6	14.5	132
	3	13.5	123
Control	1.5	13	118
5-Fluorouracil	0	11 (2)	-
	60	20 (2)	184 (2)
1,4-Bis[(3-dimethylaminocrotonyl]-amino]-5,8-dihydroxy-anthraquinone	50	15.5	129
	25	15.5	125
	12	15	125
Control	0	12	-
5-FU	60	19.5	162

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (days)	T/C x 100 (Percent)
Leuco-1,4-bis[(2-aminoethyl)amino]-5,8-dihydroxy-anthraquinone	100	11 (3)	98 (3)
	50	15 (3)	135 (3)
	25	21 (3)	181 (3)
	12	21 (3)	189 (3)
	6	19 (2)	171 (2)
	3	18.5	168
	1.36	18	164
	0.78	15.5	141
	0.39	17	155
	0.19	18	177
Control	0	11 (3)	-
5-Fluorouracil	60	19 (3)	166 (3)
Leuco-1,4-bis(3-aminopropylamino)-5,8-dihydroxy-anthraquinone	400	28	254
	200	19 (2)	161 (2)
	100	19 (2)	166 (2)
	50	17 (2)	144 (2)
	25	18 (2)	157 (2)
	12	16	133
	6	-	-
	3	-	-
	1.5	-	-
	0.75	-	-
Control	0	12 (2)	-
5-Fluorouracil	60	20 (2)	172 (2)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[2-(2-methylaminoethoxy)-amino]ethylamino]-5,8-dihydroxy-anthraquinone	200 100 50 25 12 6	10 (2) 21 (2) 21 (2) 17 (2) 16 15	84 (2) 189 (2) 190 (2) 149 (2) 145 136
Control	0	12 (2)	
5-Fluorouracil	60	18 (2)	164 (2)
Leuco-1,4-bis[2-dimethylamino-propyl-amino]-5,8-dihydroxy-anthraquinone	200 100 50 25 12 6 3 1.5 0.7	17 (3) 15 (3) 14 (3) 13 (3) 12 (3) 11 11 11 11	170 (3) 149 (3) 133 (3) 129 (3) 119 (3) 116 116 116 116
Control	0	10 (3)	
5-Fluorouracil	60	23 (3)	232 (3)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C × 100 (Percent)
1,4-Bis[2-(2-hydroxyethylamino)-ethylamino]-5,8-dihydroxy-anthraquinone, dihydrochloride	25 12 6 3 1.56 0.78 0.39 0.18 0.09 0.04 0.02	9 11 (3) 15 (5) 26 (5) 28 (5) 24 (5) 29 (5) 22 (5) 22 (3) 22 (2) 19	82 110 (3) 138 (5) 246 (5) 247 (5) 224 (5) 226 (5) 212 (5) 216 (3) 213 (2) 173
Control 5-Fluouracil	0 0.02	11 (5) 20 (5)	188 (-) -
1,4-Bis[2-(1-piperazinyl)ethylamino]-5,8-dihydroxy-anthraquinone	200 100 50 25 12 6 3 1.5 0.7	5 (3) 18 (3) 14 (3) 15 (3) 14 (3) 15 11 12 11	51 (3) 180 (3) 142 (3) 148 (3) 142 (3) 143 105 114 105
Control 5-Fluorouracil	0 60	10 (3) 21 (3)	- 202 (3)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C × 100 (Percent)
1,4-Bis[2-(methylamino)ethylamino]- -5,8-dihydroxy-anthraquinone, dihydrochloride	25	8 (2)	75 (2)
	12	14 (2)	126 (2)
	6	22 (2)	204 (2)
	3	21 (2)	196 (2)
	1.5	22 (2)	207 (2)
	0.78	18.5	176
	0.39	19.5	186
	0.19	18.5	176
	0.09	18	171
	0.04	17	162
Control	0	11 (2)	-
5-Fluorouracil	60	.9 (2)	172 (2)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[2-(2-hydroxyethylamino)-5,8-dihydroxy-anthraquinone	200	5	45
	100	5	45
	50	10 (2)	87 (2)
	25	12 (6)	113 (6)
	12	23 (6)	210 (6)
	6	27 (5)	248 (5)
	3	27 (5)	253 (5)
	1.5	26 (5)	244 (5)
	0.78	23 (4)	219 (4)
	0.39	20 (4)	188 (4)
	0.19	20 (4)	186 (4)
	0.09	21 (3)	185 (3)
	0.04	21 (3)	169 (3)
Control	0	11 (6)	-
5-Fluorouracil	60	19 (6)	174 (6)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis(4-aminobutylamino)-5,8-dihydroxy-anthraquinone	400	20	190
	300	18	171
	200	16 (2)	143 (2)
	100	14 (2)	125 (2)
	50	14	117
	25	13	108
Control	12	13	108
5-Fluorouracil	0	11 (2)	-
	60	19 (2)	176 (2)
Leuco-1,4-bis[2-(methylamino)ethyl-5,8-dihydroxy-anthraquinone	50	13 (2)	111 (2)
	25	20 (2)	170 (2)
	12	19 (2)	162 (2)
	6	19 (2)	167 (2)
	3	16 (2)	139 (2)
	1.56	14 (2)	122 (2)
Control	0.39	15	125
5-Fluorouracil	0.19	15	175
	60	19 (2)	-
			166 (2)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[2-(2-isopropylamino)-ethylamino]-5,8-dihydroxy-anthraquinone	100 50 25 12.5	8 19 17 15	73 173 155 136
Control	0	11	-
5-Fluorouracil	60	20.5	186
1,4-Bis[2-(2-aminoethylamino)ethylamino]-5,8-dihydroxy-anthraquinone	200 100 50 25	17 16 14 13	162 152 133 124
Control	0	10.5	-
5-Fluorouracil	60	17	162

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C × 100 (Percent)
Leuco-1,4-[2-[di(β-hydroxyethyl)-amino]ethylamino]-5,8-dihydroxy-anthraquinone	400	24 (2)	226 (2)
	300	16.5	165
	200	20 (2)	186 (2)
	100	18 (2)	167 (2)
	50	17 (2)	162 (2)
	25	16 (2)	150 (2)
	12	15 (2)	140 (2)
	6	14 (2)	128 (2)
	3	14.5	132
	-	-	-
Control	0	11 (2)	-
5-Fluorouracil	60	24	218
	40	18	180
1,4-Bis[2-(2-hydroxy-1-propylamino)-ethylamino]-5,8-dihydroxy-anthraquinone, dihydrochloride	25	11 (2)	99 (2)
	12	27 (2)	257 (2)
	6	27 (2)	232 (2)
	3	24 (2)	199 (2)
	1.56	19 (2)	191 (2)
	0.78	21 (2)	198 (2)
	0.39	18 (2)	170 (2)
	0.19	19	173
	0.10	18.5	168
	-	-	-
Control	0	11 (2)	-
5-Fluorouracil	60	24	218
	40	18	180

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
1,4-Bis[2-[2-(1-morpholino)ethylamino]ethylamino]-5,8-dihydroxy-anthraquinone tetrahydrochloride	200 100 50 25 12 6 3 1.56 0.78 0.39	18 (2) 20 (2) 18 (2) 19 (2) 16 (2) 16 (2) 14 (2) 14 (2) 136 (2) 15 123 12 109	166 (2) 187 (2) 172 (2) 184 (2) 148 (2) 148 (2) 136 (2) 136 123 109
Control 5-Fluorouracil	0 60 40	11 (2) 218 18 180	- -
1,4-Bis [2-(3-hydroxy-1-propylamino)-ethylamino]-5,8-dihydroxy-anthraquinone, dihydrochloride	25 12 6 3 1.56 0.78 0.39 0.19	10 (2) 24 (2) 22 (2) 22 (2) 20 (2) 20.5 19.5	99 (2) 252 (2) 226 (2) 211 (2) 210 (2) 190 (2) 205 195
Control 5-Fluorouracil	0 60 40	11 (2) 200 18	- - 164

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TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[2-(3-hydroxy-1-propyl-amino)ethylamino]-5,8-dihydroxy-anthraquinone	200	12 (2)	114 (2)
	100	34 (2)	359 (2)
	50	32 (2)	291 (2)
	25	24 (2)	214 (2)
	12	21 (2)	181 (2)
	6	19 (2)	173 (2)
	3	20 (2)	182 (2)
	1.56	19 (2)	171 (2)
	0.78	18 (2)	166 (2)
	0.39	17	155
	0.19	16	145
Control	0	11 (2)	-
5 Fluorouracil	60	19	173
	40	17	155

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (percent)
1,4-Bis[2-[di(β -hydroxyethyl)amino]-ethyl]amino]-5,8-dihydroxy-anthraquinone, dihydrochloride	600	9	90
	500	29	290
	400	30	300
	300	30	300
	200	29 (3)	291 (3)
	100	22 (3)	223 (3)
	50	22 (3)	217 (3)
	25	22 (3)	217 (3)
	12	20 (2)	213 (2)
	6	19 (2)	200 (2)
	3	19 (2)	200 (2)
	1.56	17 (2)	177 (2)
	0.78	15 (2)	156 (2)
Control	0.39	12.5	125
5-Fluorouracil	0.19	12	120
	0.10	11.5	115
	0	10 (3)	-
	60	21 (3)	.08 (3)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis [3-(2-hydroxyethylamino)-1-propylamino]-5,8-dihydroxy-anthraquinone	200	19 (2)	177 (2)
	100	27 (2)	254 (2)
	50	24 (2)	221 (2)
	25	20 (2)	189 (2)
	12	19 (2)	177 (2)
	6	19 (2)	173 (2)
Control 5-Fluorouracil	3	17 (2)	156 (2)
	1.56	16	152
	0.78	14	133
Leuco-1,4-bis[2-(2-hydroxy-1-propylamino)ethylamino]-5,8-dihydroxy-anthraquinone	200	11 (2)	-
	100	27 (2)	103 (2)
	50	26 (2)	254 (2)
	25	22 (2)	240 (2)
	12	23 (2)	205 (2)
	6	19 (2)	212 (2)
Control 5-Fluorouracil	3	2.0	177 (2)
	1.56	18.5	190
	0.78	18.5	176
Control 5-Fluorouracil	0	11 (2)	-
	40	18 (2)	170 (2)

TABLE I (continued)

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C × 100 (Percent)
1,4-Bis[3-(2-hydroxyethylamino)-1-propylamino]-5,8-dihydroxy-anthraquinone, dihydrochloride	200	4	38
	100	12 (2)	110 (2)
	50	31 (2)	284 (2)
	25	25 (2)	235 (2)
	12	22 (2)	203 (2)
	6	18 (2)	170 (2)
	3	19 (2)	172 (2)
	1.56	16 (2)	149 (2)
	0.78	15 (2)	140 (2)
	-	-	-
Control	0	11 (2)	-
5-Fluorouracil	40	18 (2)	170 (2)
1,4-Bis[2-(1-aziridino)ethylamino]-5,8-dihydroxy-anthraquinone	200	26.5	265
	100	38.5	285
	50	21.5	215
	25	20	200
	12	20.5	205
	6	18.5	185
	3	19.5	195
Control	0	11	-
S-Fluorouracil	60	20.5	205

TABLE I (continued)

Compound	Dose (mg./kg.)	Time (Days)	Median Survival (Percent)	T/C x 100
1,4-Bis[2-(2-methylaminooethylamino)-ethylamino]-5,8-dihydroxy-anthraquinone, tetrahydrochloride	100	22	220	
	50	22	220	
	25	19.5	195	
	12	17	170	
	6	16	160	
	3	13.5	135	
	1.56	13	130	
Control	0	10	-	
5-Fluorouracil	40	16	160	
1,4-Bis(2-Aminoethylamino)-5,8-dihydroxy-anthrancione, dihydrochloride	200	3	27	
	100	3	27	
	50	3	27	
	25	6	55	
	12	7	(3)	
	6	14	(3)	
	3	27	(2)	
Control	1.56	20	(2)	
5-Fluorouracil	0.78	23	(2)	222 (2)
	0.39	23	(2)	209 (2)
	0.19	21	(2)	193 (2)
	0.09	19.5		177
Control	0	11	(3)	
5-Fluorouracil	60	20	(3)	182 (3)

Lymphocytic leukemia P388 test

The procedure used is the same as for the previously described test for lymphocytic leukemia P388 except that the test compounds are administered orally at various doses rather than intraperitoneally. The results of this test with typical compounds of the present invention appear in Table II. The criterion for efficacy is $T/C \times 100 > 125\%$.

Table II
Lymphocytic Leukemia P388 Test (Oral Drug Administration)

Compound	Dose (mg./kg.)	Median Survival Time (days)	T/C x 100 (Percent)
Leuco-1,4-bis[(2-dimethylaminoethyl)amino]-5,8-dihydroxy-antrquinone	50 25 12	16 13.5 12.5	160 135 125
Control 5-Fluorouracil (administered intraperitoneally)	0 60	10 19	- 190
1,4-Bis[(2-dimethylaminoethyl)amino]-5,8-dihydroxy-ant ^r -quinone	200 100 50 25 12 6 3	6 8 10 11 17 (2) 16 15	60 70 33 110 155 (2) 139 130
Control 5-Fluorouracil (administered intraperitoneally)	0 60	11 (2) 19 (2)	- 177 (2)

Melanotic Melanoma B16

The animals used are BD₂F₁ mice, all of the same sex, weighing a minimum of 17 g. and all within a 3 g. weight range. There are normally 10 animals per test group. A one-gram portion of melanotic melanoma B16 tumor is homogenized in 10 ml. of cold balanced salt solution and a 0.5 ml. aliquot of the homogenate is implanted intraperitoneally into each of the test mice. The test compounds are administered intraperitoneally on days one, five and nine or one through nine (relative to tumor inoculation) at various doses. The animals are weighed and survivors are recorded on a regular basis for 60 days. The median survival time and the ratio of survival time for treated (T)/control (C) animals are calculated. The positive control compound is 5-fluorouracil given as a 20 or 60 mg./kg. injection. The results of this test appear in Table III. The criterion for efficacy is T/C × 100 ≥ 125%.

Table III
Melanotic Melanoma B16 Test

Compound	Dose (mg./kgs.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis [(2-dimethylaminoethyl) amino]-5,8-dihydroxy-anthraquinone	50	22	147
	25	25 (2)	156 (2)
	12	23 (2)	143 (2)
	6	21.5 (2)	137 (2)
	3	21 (2)	135 (2)
Control	0	16 (2)	-
5-Fluorouracil	20	25 (2)	161 (2)
1,4-Bis [(2-dimethylaminoethyl) amino]-5,8-dihydroxy-4-methoxy-anthraquinone	25	24.5	136
	12	28.5	158
	6	27	150
	3	25.5	142
	0	18	-
Control	0	26	144
5-Fluorouracil			

Table III (continued)
Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis[[2-(1-pyrrolidinyl)ethyl]amino]-5,8-dihydroxy-anthraquinone	100 50 25 12 6	11 22.5 21 18 19.5	73 150 140 120 130
Control	0	15	-
5-Fluorouracil	20	25.5	170
1,4-di[[2-(1-pyrrolidinyl)ethyl]amino]-5,8-dihydroxy-anthraquinone	25 12 6 3 1.5	24.5 26.5 22 20 15.5	158 171 142 129 100
Control	0	15.5	-
5-Fluorouracil	20	29.5	190

Table III (continued)
Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (days)	T/C x 100 (Percent)
1,4-Bis[(3-dimethylaminopropyl)amino]-5,8-dihydroxy-anthraquinone	25	20	125
Control	0	16	-
5-Fluorouracil	20	26.5	166
Leuco-1,4-bis[(2-aminoethyl)amino]-5,8-dihydroxy-anthraquinone			
Control			
5-Fluorouracil			

Table III. (continued)
Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
Leuco-1,4-bis(3-aminopropylamino)-5,8-dihydroxy-anthraquinone	100	37	200
	50	31	168
	25	24	130
Control 5-Fluorouracil	12	26	141
	0	18.5	-
Leuco-1,4-bis[2-(2-methylaminoethyl) amino]-5,8-dihydroxy-anthraquinone	20	29	157
	50	12.5	73
	25	35	206
Control 5-Fluorouracil	12	22.5	232
	6	28.5	168
Control 5-Fluorouracil	0	17	-
	20	30	176

Table III (continued)

Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C x 100 (Percent)
1,4-Bis[2-(1-piperazinyl)ethylamino]-5,8-dihydroxy-anthraquinone	50 25 12 6 3	34.5 30.5 26 22 20.5	203 179 153 129 121
Control	0	17	-
5-Fluorouracil	20	30	176
1,4-Bis[(2-aminoethylamino)ethylamino]-5,8-dihydroxy-anthraquinone	50 25 12 6	24 22.5 22 20	150 141 138 125
Control	0	16	-
5-Fluorouracil	20	27	169

Table III (continued)

Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (Days)	T/C × 100 (Percent)
Leuco-1,4-bis(2-dimethylaminopropylamino)-5,8-dihydroxy-anthraquinone	100 50 25 12 6	21 28.5 24.5 20.5 19.5	124 168 144 121 115
Control	0	17	-
5-Fluorouracil	20	30	176
1,4-Bis[2-(2-hydroxyethylamino)ethylamino]-5,8-dihydroxy-anthraquinone, dihydrochloride	12 6 3 2 1.5 1 0.78 0.39 0.25 0.19 0.12 0.09 0.06 0.04	11 16 (5) 39 (5) 4 55 (8) 60 44 (8) 39 (8) 34.5 172 33 (7) 32.5 1.9 (3) 30 (2) 29 (3)	73 96 (5) 235 (5) 170 219 (8) 300 238 (8) 212 (8) 172 170 (7) 162 147 (3) 142 (2) 141 (3)
Control	0	19 (9)	-
5-Fluorouracil	60 20	24 (4) 27 (5)	144 (4) 155 (5)

Table III (continued)

Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (days)	T/C x 100 (Percent)
Leuco-1,4-bis[2-(2-isopropylamino)ethylamino]-5,8-dihydroxy-anthraquinone	50	6.5	39
	25	31	188
	12	30	182
Control 5-Fluorouracil	6	25	151
	0	16.5	-
1,4-Bis[2-(methylamino)ethylamino]-5,8-dihydroxy-anthraquinone, dihydrochloride	20	16.5	100
	12	11.5	59
	6	18	96 (-)
	3	49	281 (-)
	1.5	40	235 (2)
	0.75	37	215 (2)
	0.39	31	182 (2)
	0.19	29.5	151
Control 5-Fluorouracil	0	17.5 (2)	-
	60	25	128
	20	27.5	172

Table III (continued).
Melanotic Melanoma B16 Test

	Compound	Dose (mg./kg.)	Median Survival Time (days)	T/C x 100 (Percent)
Leuco-1,4-bis(4-aminobutylamino)-5,8-dihydroxy-anthraquinone	100	21	124	
	50	19 (2)	106 (2)	
	25	18.5 (2)	106 (2)	
	12	17 (2)	99 (2)	
	6	17.5	97	
Control				
5-Fluorouracil	1	0	18 (2)	-
		20	30 (2)	169 (2)
Leuco-1,4-bis [2-(2-hydroxyethylamino)ethylamino]-5,8-dihydroxy-anthraquinone	6	9 (2)	-	(2)
	3	21 (2)	126 (2)	
	1.5	29 (2)	174 (2)	
	0.75	29 (2)	175 (2)	
	0.37	23 (2)	139 (2)	
	0.18	23.5	138	
Control				
5-Fluorouracil	0	16.5 (2)	-	
	20	29 (2)	174 (2)	

Table III (continued)

Melanotic Melanoma B16 Test

Compound	Dose (mg./kg.)	Median Survival Time (days)	T/C x 100 (Percent)
Leuco-1,4-bis[2-(methylamino)ethylamino]-5,8-dihydroxy-anthraquinone	50 25 12 6 3 1.5 0.7	5 5.5 29 (2) 33 (2) 31 36 27.5	32 35 187 (2) 208 (2) 194 225 172
Control	0	16 (2)	-
5-Fluorouracil	20 28	28 (2)	180 (2)

Ridgway Osteogenic Sarcoma

The animals used are AKD₂F₁/J mice, all of the same sex, weighing a minimum of 17 g. and all within a three-gram weight range. There are normally 8 animals per test group. The tumor is administered subcutaneously by trocar as five 2 mm. fragments per mouse. The test compounds are administered intraperitoneally every 4 days for a total of 6 inoculations beginning on day 15 (relative to tumor inoculation) at various doses. The animals are weighed and survivors are recorded on a regular basis for 90 days. The regression of tumors is recorded in all test animals. Table IV gives the result of this test in terms of the percentage of animals showing tumor regression.

Table IV
Ridgway Osteogenic Sarcoma

Compound	Dose (mg./kg.)	Per Group (mm.)	1 Day Before Therapy	No. Mice Tumor Tumors/No. Survivors	7 Days After Therapy Stopped (mm.) ²	63 Days After Therapy Stopped		
						% Inhibition	% Showing 50% Tumor Regression	Median Survival (Days)
						Tumor Growth	0	T/C (Percent)
Placebo	-	8	64	0/5	1189		0	44.5
1,4-Bis[(2-dimethylaminoethyl)-amino]5,8-dihydroxy-anthraquinone	100	7	77	2/5	52	96	28	48
	50	8	68	2/6	263	78	25	92.5
	25	8	82	0/8	653	41	0	78
	12	7	84	0/3	470	61	0	175
	6	7	83	0/6	960	19	0	83
								129
Melpharate	25	3	51	1/6	546	54	12	52.5
	12	8	52	0/5	916	23	0	49
	6	8	52	0/4	758	36	0	46
Vincristine	1.5	8	42	4/4	0	100	100	68
	1.0	6	99	6/6	0	100	100	85
	0.5	7	94	4/7	77	93	57	83

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THAT the foregoing results show the efficacy of the named compounds in inhibiting the growth of transplanted mouse tumors;
Further deponent sayeth not.

Roslyn E. Wallace
ROSLYN E. WALLACE

Sworn to and subscribed before me this 15th day of
December 1980.

Kimberly E. Miner
NOTARY PUBLIC
KIMBERLY E. MINER
Notary Public State of N. Y.
No. 4-272435
Residing in Rockland County
Commission Expires March 30, 1981